

Reaction of Drought-Tolerant Soybean Genotypes to *Macrophomina phaseolina*

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Abstract

Charcoal rot caused by *Macrophomina phaseolina* is a common disease of many crops including common bean and soybean. Incidence and severity of charcoal rot are enhanced when plants are drought stressed. Resistance to this pathogen in some common bean genotypes was associated with drought tolerance. Resistance to *M. phaseolina* among soybean genotypes has not been identified, although a few have been rated moderately resistant based on less root tissue colonization by this pathogen compared to other genotypes. A few soybean genotypes have been rated as slow-wilt or drought-tolerant. The reaction of drought-tolerant soybean to *M. phaseolina* compared to intolerant or drought-sensitive genotypes has not been determined. Our objective was to determine if there were differences in root colonization by *M. phaseolina* between drought-tolerant and drought-sensitive soybean genotypes. Drought tolerance of the soybean genotypes and root colonization by *M. phaseolina* at the R6 and R8 stages of growth were not related in this study. Some drought-tolerant soybean genotypes may resist root colonization by *M. phaseolina*, but our results suggest that this is not true for all drought-tolerant genotypes.

Introduction

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goidanich, is a fairly common disease in many parts of the world. This pathogen is distributed widely and attacks many different plant species including soybean (*Glycine max* L.) (17). When damage by this pathogen to soybean is severe, a light gray or silvery discoloration of the epidermal and subepidermal tissues develops in the taproot and lower part of the stem (Fig. 1) and leaflets first yellow and then wilt and turn brown but remain attached to the petioles (Fig. 2). Estimates of soybean yield suppression due to charcoal rot in the United States were 1.98 million metric tons in 2003, 0.28 ton in 2004, and 0.49 ton in 2005 (19). Yield suppression due to this disease is enhanced by drought, and the differences in soybean yield suppression due to charcoal rot in the United States among years were due to differences in drought among years.



Fig. 1. Lower soybean stem discoloration due to *Macrophomina phaseolina* colonization.



Fig. 2. Soybean plants damaged and killed by charcoal rot.

Drought greatly influences the incidence and severity of charcoal rot in grain sorghum (5), cotton (8), and sunflower (2). Colonization of soybean roots by *M. phaseolina* at the R8 stage of growth was significantly greater in non-irrigated than full-season irrigated soybean and was significantly greater in soybean irrigated up to but not beyond the R2 growth stage compared to full-season irrigated soybean (9).

Management strategies for charcoal rot of soybean are limited, and these strategies do not completely protect soybean against *M. phaseolina*. Cotton and corn are hosts of this pathogen, but a 3-year rotation with cotton caused the soil population density of this pathogen to decline (7), and a 3-year rotation with corn reduced soybean root colonization by *M. phaseolina* (11). Other management strategies include plant culture methods to minimize drought stress such as reduced plant populations, management of planting dates and cultivar selection to avoid mid-season drought stress (3), and irrigation (9). Tillage did not affect charcoal rot; planting conventional tillage may reduce soil population densities of *M. phaseolina*, but infection still occurred (1,18). These strategies have not been sufficient to protect soybean against charcoal rot as shown by an increase in yield suppression in the US from 336,000 tons in 1996 to 496,000 tons in 2005 (19,20).

Soybean cultivars and lines (genotypes) highly resistant to charcoal rot have not been identified. Soybean genotypes vary in root tissue colonization by *M. phaseolina*, and this is the commonly accepted method to determine genotype reaction to *M. phaseolina* (4,14,16). Recently, Paris et al (12) released a genotype that they rated moderately resistant to charcoal rot because root tissue colonization by *M. phaseolina* was lower for this genotype than most others.

Pastor-Corrales and Abawi (13) observed that resistance in common beans to *M. phaseolina* was associated with drought tolerance. Some soybean genotypes have been categorized as drought-tolerant (15). The reaction of drought-tolerant compared to drought-sensitive soybean genotypes to *M. phaseolina* is not known. Our objective was to determine if there were differences in root colonization by *M. phaseolina* between drought-tolerant and drought-sensitive soybean genotypes.

Evaluation of Soybean Genotypes for Root Colonization by *Macrophomina phaseolina*

An experiment was established from 2003 to 2005 to compare the root colonization by *M. phaseolina* among select drought-tolerant and drought-sensitive soybean genotypes. The phenotype of soybean PI 416937 is slow wilt under drought stress (i.e., drought-tolerant) (15), and the phenotypes of PI 471938, N987165, N987265, and N987288 are also slow wilt (T. Carter, *personnel communication*). Delta King 4868 and Hutcheson were selected for comparison with these genotypes because they are of similar maturity group to the drought-tolerant genotypes, and these two are not drought-tolerant (G. Shannon, *personnel communication*). The soil was a sandy loam with 6.3% clay, 37.2% silt, and 56.5% sand, and the field had been planted to soybean the three previous years. Prior to planting each year, the field was disked twice, and row

beds (75-cm spacing) were formed. The top 10 cm of each bed was pushed off just prior to planting to form a flat-top ridge. Recommended agronomic practices from the University of Missouri Extension were used for weed control, and fertilization. Each four-row plot was 10 m long. Planting dates were mid-May in all years. The genotypes were planted at 26 seed/m. Plots were moved to an adjacent area of the field each year to avoid uneven distribution of soil population densities of *M. phaseolina* due to the previous years treatments. Drought developed during 2003 and 2005, but rain was abundant during 2004. Total rain during July 1 to October 1 was 113 mm during 2003, 200 mm during 2004, and 100 mm during 2005. All genotypes were in the R1 to R8 stages of growth during this time. Data from 2004 were not used because drought stress did not occur and root tissue colonization by *M. phaseolina* was similar among genotypes (*data not shown*). The center two rows of each plot were harvested by combine. The seed were weighed and tested for moisture to determine yield at 13% moisture. A randomized complete block design with eight replications was employed to evaluate seven soybean genotypes.

At planting, soil samples were collected from each plot to estimate densities of microsclerotia (18). Soil samples were air-dried and then passed through a 45- μ m-mesh sieve. A 5-g portion of the sieved soil was suspended in a 500-ml flask containing 250 ml of 0.5% sodium hypochlorite. The flask containing the suspension was placed on a rotating shaker (120 cycles/min) for 10 min. The suspension was then poured onto a 325- μ m-mesh sieve, and the debris was rinsed with tap water for 30 s. The residue on the sieve was transferred to a 250-ml flask, and 100 ml of the selective medium Cholorneb-Mercury-Rose Bengal agar (CMRB) was added. The suspension was gently swirled and divided among five petri plates. The petri plates were incubated in the dark at 33°C for 7 days. Microsclerotia densities were calculated from the number of colony-forming units on the plates and adjusted to a per gram of air-dried soil basis. The colonies were identified as a ring of fluffy white mycelium surrounding a central area with black sclerotia (18).

Late-season colonization of soybean roots by *M. phaseolina* was investigated during 2003 and 2005. Six arbitrarily selected plants were removed from the outside two rows of each plot when the plants were at the R6 and R8 stages of growth (6). Roots were washed to remove soil, and they were surfaced disinfested in 0.5% sodium hypochlorite for 1 min, and rinsed in water for 60 s. The roots were dried for 24 h at 28°C to eliminate further tissue colonization by the fungus, and preserved for determination of *M. phaseolina* microsclerotial densities (9). Once dried, the roots were ground with a Wiley mill equipped with a 0.2-mm screen. A 0.5-g portion of the root tissue was transferred to a 250-ml flask, 100 ml of the CMRB was added, and incubated in a 45°C water bath for 20 min. The contents were poured into 10 petri plates, and incubated in the dark at 33°C for 7 days before the colonies of *M. phaseolina* were counted.

Data were log transformed, and ANOVA was used to analyze all data. Mean separation was by an *F* test protected LSD (10). Values for cfu/g dry root are presented in the results rather than log cfu/g dry root. Charcoal rot symptoms on stems and foliage did not develop.

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The soil population densities of *M. phaseolina* at planting time each year were similar among plots. They were 250 cfu/g dry soil in 2003, 500 cfu/g dry soil in 2004, and 340 cfu/g dry soil in 2005.

Root colonization by *M. phaseolina* differed among genotypes at the R6 and at the R8 stages of growth (Table 1). Significant interactions were not observed between years and genotypes for root colonization at the R6 and R8 stages of growth. At the R6 growth stage root tissue colonization was significantly lower for PI 416937 (2640 cfu/g root) and N987288 (1525 cfu/g root) than Hutcheson (4000 cfu/g root); however, by growth stage R8, only DK4868 had less root colonization than Hutcheson (Table 2). These results agree with other reports (4,9,12,14,16) that there is variation in root tissue colonization by this pathogen among genotypes.

Table 1. Analysis of variance for colony forming units of *Macrophomina phaseolina* (log cfu/g root) at the R6 and R8 stages of growth for year and soybean genotypes.*

Source of variation	<i>P</i> > <i>F</i>	
	R6	R8
Year (Y)	0.2958	0.0198
Genotype (G)	0.0101	0.0028
Y × G	0.0669	0.0975

* Years were 2003 and 2005, and genotypes were PI 416937, PI 471938, N 987165, N 987265, N 987288, Delta King 4868, and Hutcheson.

Table 2. Root colonization of soybean genotypes by *Macrophomina phaseolina* (cfu/g root) at the R8 stage of growth for 2003 and 2005.

Soybean genotype	2003	2005
PI 416937	11660 a*	8320 a
PI 471938	13210 a	9330 a
N 987165	10882 a	7826 a
N 987265	11710 a	8810 a
N 987288	12115 a	9220 a
Delta King 4868	7680 b	5300 b
Hutcheson	10250 a	8180 a

* Values within a column followed by the same letter are not significantly different (*P* = 0.05).

Yield differed among genotypes (*P* < 0.0001) and among years (*P* = 0.008). Because there was an interaction between genotype and year (*P* = 0.002), the yield data are presented by year (Table 3). Soil moisture was more abundant in 2004 than during 2003 and 2005, and most genotypes yielded more during 2004 than other years. During 2004 the drought-sensitive genotypes DK4868 and Hutcheson yielded significantly greater than the other genotypes (Table 3). Soil moisture was less abundant during 2003 and 2005 than 2004, and yields were similar these years for drought-tolerant PI 987165, N987265, and N987288 and drought-sensitive DK4868 and Hutcheson.

Table 3. Soybean genotypes and year effects on yield (kg/ha).

Genotypes	Year		
	2003	2004	2005
416937	1626 c*	1734 d	1492 c
471938	2466 b	3104 c	1398 c
N987165	3292 a	3608 b	2318 ab
N987265	2560 b	3460 bc	2338 ab
N987288	2775 ab	3333 bc	2600 a
DK 4868	2768 ab	4213 a	2157 ab
Hutcheson	3138 a	4347 a	2029 b

* Values within columns followed by the same letter are not significantly different (*P* = 0.05).

Drought tolerance of the soybean genotypes and colonization by *M. phaseolina* at the R6 and R8 stages of growth were not related in this study. For example, root colonization by *M. phaseolina* at R8 was significantly lower for the drought-sensitive DK 4868 than other genotypes and was greater for the drought-tolerant PI 471938 than others. Some drought-tolerant soybean genotypes may resist root colonization by *M. phaseolina*, but our results suggest that this is not true for all drought-tolerant genotypes. Additional research is

needed to determine whether the effects of drought and infection by *M. phaseolina* are additive, synergistic, or independent.

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